Good Things Come in Small Packages; Molecular Techniques for Picoeukaryote Identification

Jessica Nolan
York College of Pennsylvania
Country Club Road
York, PA 17405

When most people think about life in the ocean, they think of large organisms such as whales, fish, and sea turtles. However, scientists also study life on the opposite end of the size spectrum—some of the smallest organisms in the ocean. Phytoplankton are single-celled organisms in the ocean that photosynthesize. Picophytoplankton are the smallest of the phytoplankton, less than 2–3 micrometers (μm) in size. To appreciate how small this is, we can compare it to the size of a single grain of sand (Figure 1). A normal phytoplankton cell might be 30 μm in size, a third the size of a grain of sand. A picophytoplankton cell can be 100 times smaller than a single grain of sand!

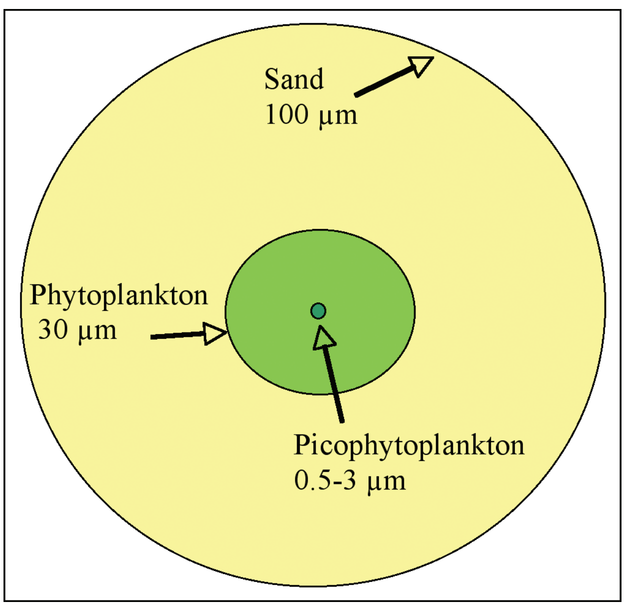
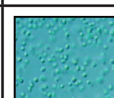
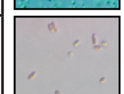
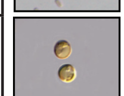


Figure 1. Comparison of the size of a grain of sand, a typical phytoplankton, and a picophytoplankton.

There are both advantages and disadvantages to being so small. The most important benefit is that small cells can acquire resources more easily. Phytoplankton need light and nutrients, just like plants on land. Their small size means they are more likely to be photoinhibited (exposed to too much light) and they have less available space to store nutrients. The advantages of a small size, however, appear to outweigh the disadvantages [1].

Picophytoplankton can be either prokaryotic (simple, without a nuclear membrane) or eukaryotic (complex, with a nuclear membrane). The prokaryotic picophytoplankton are dominated by two genera, *Prochlorococcus* and *Synechococcus*. Both were discovered relatively recently—*Synechococcus* in 1979 [2], and *Prochlorococcus* in 1988 [3]. Before these discoveries, the importance of picophytoplankton was not realized. *Prochlorococcus* is the smallest known photosynthetic organism and is thought to be the most abundant phytoplankton on Earth (Table 1; see review [4]). *Synechococcus* is a bit larger in size and is found from the North Pole to the South Pole (see review [5]).

TABLE 1
Comparison of Picophytoplankton

Type	Picture	Cell size (μm)	Cell number (cells mL^{-1})
Prochlorococcus		0.5–0.7	100,000
Synechococcus		0.6–1.6	10,000
Picoeukaryote		0.8–2	1,000

Much research has been done to understand *Prochlorococcus* and *Synechococcus* and their roles in the environment. For example, it is now believed that they contribute significantly to the amount of photosynthesis in the ocean [4, 5]. Researchers have even sequenced the complete genome from both [6, 7]. These discoveries have revealed an entire realm of organisms we did not even know existed and have revolutionized how we think about phytoplankton in the ocean.

The eukaryotic component of the picophytoplankton is very different. Often referred to as picoeukaryotes, they are larger in size and fewer in number than either of the prokaryotes (Table 1; [8]). They are also more diverse and much less studied. Currently, there are picoeukaryotes known from 10 algal classes, however, new species are being found each year. For much of the world, we don't know how many species there are, which are most common, how they are distributed, and how they change seasonally. We don't know what niches or roles they play in the environment, how much they photosynthesize, or who eats them. These are exciting questions that desperately need to be studied.

Measuring Diversity

One of the primary reasons that relatively little research has been done with picoeukaryotes is the difficulty associated with identifying them. Traditionally, phytoplankton have been identified by distinguishing characteristics using a microscope, however, most picoeukaryotes are small, round, and green or brown in color (Figure 2). This makes them difficult or impossible to tell apart using a compound microscope, or even a more specialized research microscope (such as an epifluorescence or electron microscope). A phytoplankton cell can also be selected and grown in the lab in order to study a specific species in more detail. This method tends to be selective as some species grow better under a certain set of conditions (e.g., light, temperature, or nutrients). Most (90–99%) phytoplankton cannot be cultured in the laboratory. Those species that grow well in the lab are not always the same species that are found most often in the ocean [9]. Instead, scientists have been turning to molecular techniques to help investigate the diversity of picoeukaryotes.

These molecular techniques are based on knowledge that DNA sequences vary between different taxa (for example, different classes, genera, or even species). Often, the first step is to look for a section of DNA[1] that is unique to the organism or group of interest. Frequently, researchers use a gene that codes for a subunit of the ribosome (ribosomes are the machinery for making proteins). Because species have ribosomes, they all must have this gene. In eukaryotes, this gene is referred to as the 18S ribosomal RNA[2] gene. For example, if a phytoplankton cell had the sequence ACG TCC TTG TTC GAC GCT[3] in its 18S RNA gene, you would know this species was in the class Pelagophyceae (Figure 3) as that sequence is unique to Pelagophyceae [10].

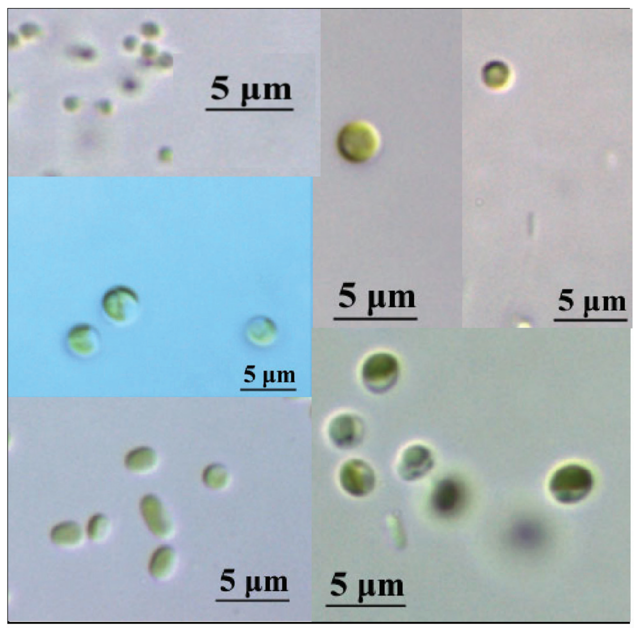


Figure 2. A variety of picoeukaryotes. Note the similar shape and color. (Pictures courtesy of Provasoli-Guillard National Center for Culture of Marine Phytoplankton—Bigelow Laboratory for Ocean Sciences)

1 DNA: deoxyribonucleic acid
2 RNA: ribonucleic acid
3 The major amino acid building blocks of DNA and RNA are Cytosine (C), Guanine (G), Adenine (A), and Thymine (T).

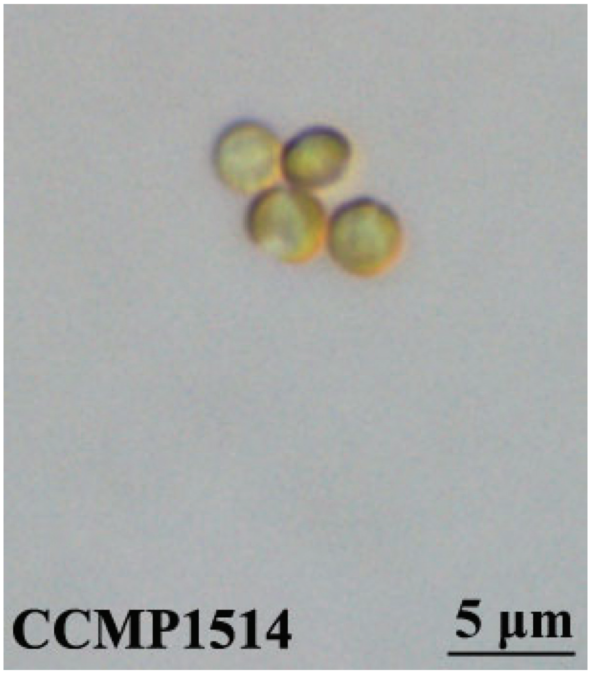


Figure 3. The class Pelagophyceae can be identified using molecular techniques by the sequence ACG TCC TTG TTC GAC GCT in its 18S RNA gene (Pictures courtesy of Provasoli-Guillard National Center for Culture of Marine Phytoplankton—Bigelow Laboratory for Ocean Sciences).

Once a researcher has decided on which segment of DNA they will use, there are several techniques they can employ to investigate the diversity in the ocean. Some researchers have created a "library" of a particular gene, like the 18S RNA gene discussed above. The library is used to catalog many of the picoeukaryote sequences found in a particular location [11, 12]. To create a library, DNA is extracted from the picoeukaryotes. A section of DNA is then inserted into a bacterium cell. As the bacteria reproduce, they make more and more copies of the sequence. The section of DNA can then be isolated from the bacteria and sequenced. This can be repeated many times until a collection of DNA sequences exists in the library. This technique gives scientists many sequences from the environment, but it is also time consuming and there are some biases.

An alternative is to get a snapshot view of the entire picoeukaryote community using a procedure called denaturing gradient gel electrophoresis (DGGE) [13, 14]. In this method, DNA from the environment is amplified so there are many copies. The DNA is broken down, or denatured, by a gradient of chemicals in a gel. DNA with different sequences will break down at different times, leaving a series of bands on the gel (Figure 4). Each of the bands corresponds to a different sequence and thus, different taxa. The bands can be cut out and sequenced, then compared to known species. The major advantage of this technique is that it is possible to run several samples on a single gel to compare different locations, depths, or seasons.

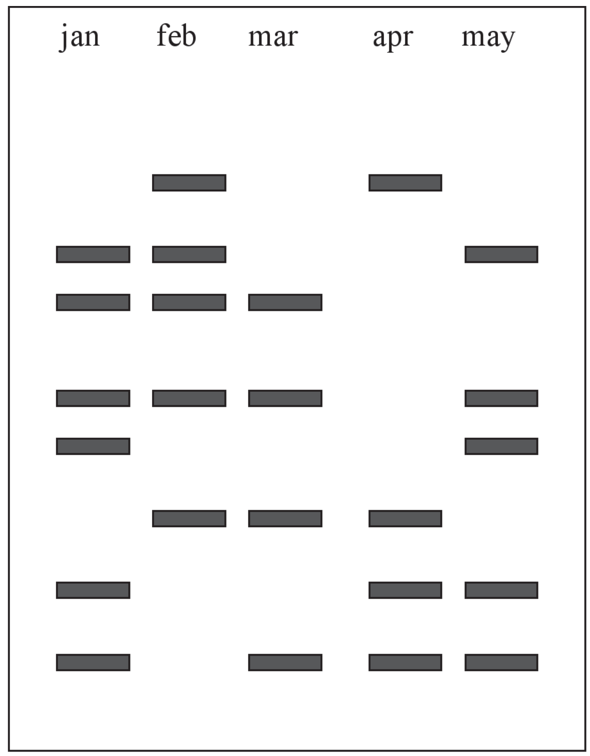


Figure 4. An example of a hypothetical DGGE gel. Individual bands represent unique DNA sequences. In this example, there are samples shown from five months. In January, six different taxa were found, while in March, only four taxa were found.

A final method used to investigate diversity is to design probes that are specific to picoeukaryote groups [15]. The probes are small segments of DNA that can be added to a sample. The probes attach to cells that have complementary segments of DNA. When put under a special microscope, the cells glow and can be counted. This technique, called fluorescent *in situ* hybridization (FISH), is the only method that allows a researcher to count how many cells of a particular species are in a sample.

Several molecular studies have been completed from the Pacific [12, 16], Southern Ocean [11, 17], North Atlantic [11], Mediterranean Sea [11, 9], and English Channel [18]. All studies noted that a single class of picoeukaryotes, Prasinophyceae, made up a large portion of the phytoplankton sequences retrieved. In some cases, a single genus dominated the samples collected [19]. Sequences from a number of other classes were also frequently found. For example, prymnesiophytes seem to be very important in the open ocean, but there is much less data from these regions as they are much harder to study than the coasts [12]. There are many places around the world where the picoeukaryote diversity has not been examined. Will new areas also be dominated by a single class of picophytoplankton? Or will we discover greater diversity as we continue to investigate new locations? Picoeukaryotes are an expanding area of study. In the next several years, there will undoubtedly be a number of studies that will enlarge our knowledge and may even revolutionize how we think of picoeukaryotes and their role in the environment.

Acknowledgments

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Additional Reading

E.F. Delong, "A plentitude of ocean life," *Natural History,* vol. 112, pp. 40–46, 2003.

S. Nadis, "Insights: The cells that rule the seas," *Scientific American,* vol. 289, pp. 52–53, 2003.

Web Sites

http://ccmp.bigelow.org

The Provasoli-Guillard National Center for Culture of Marine Phytoplankton—Bigelow Laboratory for Ocean Science. This site provides information on many of the picophytoplankton currently in culture (with pictures and molecular information when available) and also includes instructions for growing phytoplankton in a lab.

http://europa.eu.int/comm/research/news-centre/en/env/02-10-env01.html

European Research News Centre, "The Lilliputians of the Plankton World"—Much of the research on picoplankton has been completed by a group of European labs as part of the PICODIV program. This site provides an overview of their work.

http://www.jgi.doe.gov/whoweare/microbialgenome.html

DOE Joint Genome Institute, Microbial Genomic page—The genomes of two picophytoplankton, *Prochlorococcus* and *Synechococcus,* have been sequenced and this is the official Web site.

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Glossary

Eukaryotic—pertaining to a complex cell with a nuclear membrane.

Niche—the ecological role of an organism within the community.

Photosynthesis—incorporation of CO_2 into organic molecules.

Prokaryotic—pertaining to a simple cell without a nuclear membrane.

Ribosome—the site of protein synthesis within a cell.

Taxa—a classification or grouping of organisms (i.e., kingdom, phylum, class, order, family, genus, species).

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Jessica K. Nolan: Jessica Nolan completed her undergraduate degree at the University of New England in Biddeford, ME and her graduate work at Scripps Institution of Oceanography in La Jolla, CA. During her time as an undergraduate and graduate student, she had the amazing opportunity to travel around the world conducting research. She travelled to Alaska and twice to the Indian Ocean. Ms Nolan loves research cruises as they provide the opportunity to meet other researchers and see what they are doing, see all types of incredible organisms (from bioluminescent phytoplankton to whales), and explore the ecology and culture of a new country. She's currently an Assistant Professor of Biology at York College of Pennsylvania. Her main research interests involve picophytoplankton diversity, ecology, and optics; however, with her students, she's done research on a wide range of marine and freshwater organisms.